

CLAIMS

1. A method for the *in vitro* determination of cellular uptake of exogenous or endogenous substances in a cell sample, which method comprises:
 - 5 1) selecting a suitable shift agent (SA) and nucleus combination for the measurement of cellular uptake of the exogenous or endogenous substance under investigation, through MAS-NMR spectroscopy;
 - 2) determining the cellular compartment/s (CC/s) in which said exogenous or endogenous substance distributes, through MAS-NMR spectroscopy; and
 - 10 3) measuring the compartmental concentration of the said exogenous or endogenous substance.
2. A method according to claim 1 wherein step 1) is carried out by:
 - 15 a) identifying a set of possible SA candidates for said SA and nucleus combination, on the basis of the LIS produced on at least one NMR signal belonging to said exogenous or endogenous substance;
 - b) identifying a set of possible candidates for said SA, on the basis of the CC/s in which they distribute; and
 - c) selecting said SA and nucleus combination, on the basis of the information
20 gathered from steps (a) and (b).
3. A method according to claim 1 wherein step 2) is carried out by:
 - d) acquiring the MAS-NMR spectrum of the *in vitro* sample containing the exogenous or endogenous substance under investigation and determining the
25 marker^{EXO} or marker^{ENDO} signal/s;
 - e) adding a suitable amount of the selected SA to the above *in vitro* sample, so as to induce a significant LIS of marker^{EXO} or of marker^{ENDO} signal/s, and re-acquiring the same MAS-NMR spectrum; and
 - f) comparing the marker^{EXO} or the marker^{ENDO} signal/s of steps (d) and (e)
30 and determining in which Cellular Compartment the exogenous or endogenous substance is present.

4. A method according to claim 1 for the in vitro determination of cellular uptake of exogenous substances.

5. A method according to claim 4 wherein the exogenous substance is any substance
5 not naturally occurring in a biological sample.

6. A method according to claim 5 wherein the exogenous substance comprises exogenous organic substances or exogenous metals or metal ions which NMR signals can be observed.

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7. A method according to claim 6 wherein the exogenous substance is selected from the group consisting of: drugs for human and veterinary use, diagnostic and therapeutics agents, contrast agents for imaging techniques, radio-sensitizers for photodynamic and neutron capture therapy, pesticides, herbicides, fertilizers, food additives, preservatives,
15 cosmetics, colorants, waste products, pollutants, and chemicals.

8. A method according to claim 1 wherein the endogenous substance comprises any substance resulting from normal or pathological biochemical processes of cells and tissues.

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9. A method according to claim 8 wherein the endogenous substance is selected from the group consisting of natural carbohydrates, urea, lactate, citrate, acetate, carbonate, malonate, choline, creatine, phosphate, piruvate and natural amino acids.

25 10. A method according to any previous claim wherein the SA is selected from compounds containing a metal ion of the lanthanide group including: Ce^{3+} ; Pr^{3+} ; Nd^{3+} ; Pm^{3+} ; Sm^{3+} ; Eu^{3+} ; Tb^{3+} ; Dy^{3+} ; Ho^{3+} ; Er^{3+} ; Tm^{3+} ; and Yb^{3+} .

11. A method according to claim 10 wherein the SA comprises lanthanide complexes of
30 ligands selected from: EDTA (ethylenediaminetetracetic acid); PCTA (3,6,9,15-tetraazabicyclo-[9.3.1]-pentadeca-1(15)11,13-triene-3,6,9-tris (methane phosphonic acid); BOPTA ((4RS)-[4-carboxy-5,8,11-tris (carboxymethyl)-1-phenyl-2-oxa-5,8,11-

triazatridecan-13-oic acid]) or derivatives thereof; DTPA (diethylenetriamine pentaacetic acid) or derivatives thereof; DOTA (1,4,7,10-tetraazocyclo-dodecane-N,N',N'',N'''-tetraacetic acid) or derivatives thereof; DO3A (1,4,7,10-tetra azacyclododecane-1,4,7-triacetic acid) or derivatives thereof; DOTP (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis (methane phosphonic) acid or derivatives thereof; and ([3 β (R),5 β ,12 α]-3-[[4-bis[2-bis(carboxymethyl)amino]-ethyl]amino]-4-carboxy-1-oxobutyl]amino]-12-hydroxycholan-24-oic acid).

12. A method according to any previous claim wherein the cell sample is selected from human or animal cells, cells cultures, tissues and organ cells, vegetal cells, part of trunks, leaves and food cells of both animal or vegetal origin.

13. The method of claim 1 for use in the fields of medicine, diagnostics, photodynamic and neutron capture therapy, pharmacology and pharmacokinetics, toxicology, cosmetics, food preservation, and botanics.

14. Use of the method according to any one of the preceding claims for the determination of the kinetic parameters of cellular uptake.